OBSERVATIONS ON *TRINEMA LINEARE* PENARD (TESTACEA: PROTOZOA)

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OBSERVATIONS ON *TRINEMA LINEARE* PENARD (TESTACEA : PROTOZOA)

By R. H. HEDLEY AND C. G. OGDEN

CONTENTS

SYNOPSIS

Trinema lineare, a geographically widespread, fresh-water, moss and soil inhabiting testacean, has been established in clonal culture with a doubling time of between 72 and 78 hours. Full descriptions are given of the siliceous shell and the fine structure of the vegetative stage. Ultrastructural features of special interest include the presence of microbodies, and a microtubule-organizing-centre associated with the nucleus at prophase.

INTRODUCTION

THE five known species of *Trinema* Dujardin, 1841, are amongst the most widely reported rhizopods to be found in soil, sphagnum moss and fresh-water habitats. Very little is known of their biology and there has been no previous report of the cytoplasmic ultrastructure. The present paper is an account of the fine structure of cultured specimens of *Trinema lineare* Penard, 1890, isolated from a sample of moss and soil from Cliffe Marshes, Rochester, Kent, in February, 1970. It is the second of a series of papers devoted to testate amoebae aimed at providing a fuller understanding of their biology, and a fuller appreciation of the significance and nature of the siliceous shell. The first paper in the series was devoted to a detailed account of *Euglypha rotunda* – a widely distributed species (Hedley and Ogden, 1973).

Trinema belongs to the family Euglyphidae and the classification adopted here is that proposed by Loeblich and Tappan (1961) :

Class	*RHIZOPODEA*	Von Siebold, 1845
Subclass	*FILOSIA*	Leidy, 1879
Order	GROMIDA	Claparède and Lachmann, 1859
Superfamily	**EUGLYPHACEA**	Loeblich and Tappan, 1961

Family **EUGLYPHIDAE** Wallich, 1864;
test hyaline, symmetrical, elongate, composed of rounded siliceous scales, aperture rounded or elongate : one nucleus.

Previous work – biology

Leidy (1879) examined several testate amoebae from North America and suggested that the cytoplasmic structures of *Trinema* were similar to those in *Euglypha*, in that each had a single nucleus and two contractile vacuoles. Penard (1902) observed that when the animals were inactive, or in the vegetative phase of their life cycle, the granular zone and nucleus appeared to be distinct. Dunkerly (1923) reported that reserve shell-plates were arranged around the nucleus, and suggested that certain dark granules were chromidia. Chardez (1960) noted that the cytoplasm did not fill the shell cavity and that it appeared to be attached only at the apertural collar.

Both Penard (1902) and Leidy (1879) described the pseudopodia as very fine, usually two or three and occasionally six in number. According to Leidy (1879) when the animal moves, the body is inclined so that the aperture faces anterior and down, while the fundus points backwards and up.

During binary fission, Penard (1902) observed that the animals are diametrically opposed whereas at conjugation they appear to be directly apposed. Chardez (1960) observed the part of the reproductive cycle when clear cytoplasm passed into the daughter shell, until the moment that the daughter cell became packed with agitated vacuoles. The cytoplasmic volume attained full size within 20 minutes. Chardez (1960) also reported the formation of a cyst with two nuclei as a phenomenon of conjugation, in which the cytoplasm of the two conjugants become joined in one shell, the empty shell remaining provisionally united. Similar united individuals, with one shell empty and the other containing cytoplasm and two nuclei, were observed by Dunkerly (1923) who considered this to be a stage of encystation, after which the cytoplasm contracted, the chromidia disappeared – at least as staining bodies – and finally the nuclei fused. Dunkerly (1923) also suggested that the chromidia were used up during encystation as reserve food material.

As a result of an ecological study of a Netherland fen, de Graff (1956) reported that *Trinema enchelys* and *T. lineare* show an optimum distribution in moderate dry mosses, but were found in most kinds of biotopes, only *T. lineare* avoiding the drier mosses. In a review of soil protozoa, Stout and Heal (1967) described *T. lineare* as an ubiquitous species, found in both organic and mineral topsoils, and having the same pH tolerance as the common soil ciliates and flagellates. They also stated that most testaceans – with some exceptions including *T. lineare* – reproduce slowly, have poor encystment mechanisms and poor tolerance to high carbon dioxide and low oxygen tensions and salinities.

Previous work – taxonomy

T. lineare was described first by Penard (1890), who considered it to be smaller and more elongate than *T. enchelys* (Ehrenberg, 1838). The same author (Penard,

1902) later redefined the size as being 16–26 μm and rarely 30 μm. Cash *et al.* (1915) redescribed three species of *Trinema*, including *T. lineare*, and one variety in a review of the *British Rhizopoda Fauna*. They listed *T. acinus* Leidy, 1879 as a synonym of *T. lineare*, but this is probably a doubtful species because Leidy (1879) described only *T. enchelys* and figured only *T. acinus*. Both Volz (1929) and Kufferath (1932) suggested that *T. lineare* was a synonym of *T. enchelys*, whereas subsequent authors have recognized both species. Hoogenraad and de Groot (1940) redescribed three species and listed some measurements of previous authors. Chardez (1956) figured numerous variations in the shape and size of the shell of both *T. enchelys* and *T. lineare*.

Thomas (1958) suggested that there are three types of shell: firstly, those with large completely overlapping plates; secondly, those with large incompletely overlapping plates with smaller plates filling the gaps; and thirdly, those with large separate plates with many small plates; the first type is seen fairly often, the second is very common whilst the last type is rare.

Bonnet and Thomas (1960) redescribed five species of *Trinema* and one variety, *T. complanatum* Penard, 1890; *T. complanatum* var. *globulosa* Chardez, 1959; *T. enchelys*; *T. galeata* (Penard, 1890); *T. lineare* and *T. penardi* Thomas and Chardez, 1958, with a list of their recorded distribution. In several publications Decloitre (1961a, b, 1964a, b, 1965a) has provided additional measurements and localities, and discussed variation seen in some testaceans. Decloitre (1962) described a new variety of *T. lineare*, *T. lineare* var. *terricola* having a ventral aperture, and more recently Stepanek (1967) described two varieties, *T. lineare* var. *globulosa* having a mouth at one side and *T. lineare*, var. *pellucida* having an elliptical mouth.

MATERIALS AND METHODS

T. lineare was isolated from a sample of moss and soil collected on Cliffe Marshes, near Rochester, Kent, in February, 1970. Crude cultures were made from small portions of this material placed in the culture liquid and kept in the laboratory at room temperature, 18–20°C. Agnotobiotic cultures were kept, in small plastic containers, on a thin substrate of agar (1 per cent agar agar in distilled water) with a sterilized wheat grain added prior to setting, and covered with a shallow layer of the culture liquid. This liquid was a 5 per cent (w/v) solution of soil extract, plus 100 mg/l^{-1} of sodium nitrate and 15 mg/l^{-1} of sodium dihydrogen orthophosphate, in distilled water.

Clonal cultures were obtained by isolating single, active animals. One such clone was subsequently used to produce working cultures. If sub-cultures are made at intervals of between three or four weeks the animals appear to feed and reproduce readily. The clone which was used to produce working cultures is now deposited at the Culture Centre of Algae and Protozoa, the Natural Environment Research Council, Cambridge, England.

Optical microscopy – The animals were examined by bright-field and phase-contrast microscopy, either alive or after fixation. Smears fixed in either Schaudinn's fluid or glutaraldehyde were stained with either borax carmine or iron haematoxylin.

Scanning electron microscopy – For morphological studies on external morphology both live animals and empty shells were used. Living specimens were fixed initially in 3 per cent glutaraldehyde in distilled water for 30 minutes. The empty shells were washed initially in several changes of distilled water. Single specimens were then passed through several changes of triple glass distilled water using either a single-hair brush or a fine-bore pipette. They were then manipulated onto a small cover-slip, previously cleaned with acetone and lint-free tissue, and allowed to dry. Dried specimens adhere well to glass but can be moved by use of a moistened single-hair brush. For the examination of individual siliceous plates, single clean specimens were placed on a fragment of cleaned cover-slip, covered with a small drop of concentrated sulphuric acid and gently heated. On evaporation of the acid the plates are liberated from the organic cement. The treated cover-slips were attached to 'Stereoscan' stubs by an electrically-conductive paint, 'Silver Dag', and then coated evenly with 10–15 nm of gold using an Edwards coating-unit with a planetary specimen holder (Harris *et al.*, 1972). The stubs were examined on a Cambridge Stereoscan Mk II at either 15 or 20 kV and the results recorded on Ilford 35 mm HP3 film.

Transmission electron microscopy – Animals were fixed at room temperature for 15 minutes in 1 per cent glutaraldyhyde in 0·05 M Sorenson's phosphate buffer plus 0·0015 M calcium chloride, followed by 10 minutes in 3 per cent glutaraldehyde in the same buffer. After several rinses in buffer, they were post-fixed in 1 per cent osmium textroxide in 0·1 M Sorenson's buffer. To facilitate subsequent handling the specimens were occasionally embedded in 1·5 per cent agar at this stage, prior to dehydration. The material was dehydrated by passage through a series of graded water/ethanol mixtures, ending in absolute ethanol and embedded in Epon 812. Sections were cut on a Porter Blum MT2 ultramicrotome using a Du Pont diamond knife, stained with alcoholic uranyl acetate and Reynold's lead citrate, and examined in an A.E.I. 6B electron microscope operating at 60 kV. The results were recorded on Ilford's 'Special Lantern Contrasty' plates.

DISTRIBUTION

T. lineare is commonly found in damp and wet mosses and various soils. A list of localities and references from which it has been recorded is given by Bonnet and Thomas (1960). Additional localities are given here based on records of several authors, namely, Hoogenraad and de Groot (1952a, b), Bonnet (1966), Decloitre (1964a, 1965a, b), Chardez (1961, 1969) and Golemansky (1971).

The following list illustrates the geographically wide distribution of *T. lineare* :

EUROPE : Great Britain, Spitzbergen, Iceland, Finland, France, Belgium, Netherlands, Germany, Italy, Czechoslovakia and Hungary.

NORTH AMERICA : United States of America, Canada and Greenland.

SOUTH AMERICA : Tristan da Cunha, Chile, Bolivia and Peru.

AFRICA :	Angola, Morocco, Congo, French Cameroons, Algiers, Guinea, South Africa and the Seychelles.
ASIA :	India, China, Siberia and Japan.
AUSTRALASIA :	Australia, New Zealand and Tasmania.
ANTARCTICA :	South Georgia and Gough Island.

MORPHOLOGY AND VARIATION

The siliceous shell is elliptical in shape through the major axis and circular through the minor axis ; it varies in length between 25 and 34·5 μm and in breadth between 14.5 and 19 μm (Pl. 1, figs. B and E). The aperture is normally circular, invaginated and situated sub-terminally, having a diameter of between 6 and 8·5 μm (Pl. 1, figs. A, B and E). Its position may vary greatly through various angles to the longitudinal axis of the shell, including being terminal. The shell is composed of three different types of siliceous plates : small, circular apertural plates (Pl. 1, figs. C and D), large, circular shell-plates (Pl. 1, fig. B) and small, oval to circular shell-plates (Pl. 1, figs. D and F).

The apertural plates are roughly circular, folded along the median line with a small central dorsal tooth (Pl. 1, figs. C and D), and vary between 1·2 and 1·5 μm in diameter. The number per specimen appears to vary between 18 and 28, but the majority of animals have between 22 and 26. The apertural plates may occasionally be moved from their marginal position either to form double rows or to be displaced out of symmetry. Usually either one or two rows of between 13 and 16, small shell-plates are arranged adjacent to the apertural plates (Pl. 1, fig. A), but these are also subject to some variation, in one instance being replaced by large shell-plates.

The large circular, slightly convex shell-plates have a diameter of between 4·0 and 5·5 μm and a thickness of between 0·12 and 0·20 μm ; whereas the small oval to circular, convex shell-plates (Pl. 1, fig. F) vary in length between 2·4 and 3·5 μm and are between 0·11 and 0·20 μm thick. *T. lineare* has approximately 50 incompletely overlapping large shell-plates with an unknown number of small shell-plates haphazardly filling the interstices, many of them being completely overlaid by the large shell-plates. This is the second and most common of the three types of shell-plate arrangement noted by Thomas (1958). Although the plates are normally arranged evenly, an occasional shell is seen in which some shell-plates are reversed, with the concave surface facing outwards (Pl. 6, fig. E).

The incidence of abnormally shaped shells (Plate 2) is usually less than 1 per cent, although as many as 10 per cent of one culture was observed to have abnormal forms. Cash *et al.* (1915) and Chardez (1970) have illustrated previously the variation in the position of the aperture and included some examples of evaginated apertures. The most frequently seen abnormal forms are a single shell possessing two apertures – an aperture being defined as an opening bordered by apertural plates. Openings in the shell due to inadequate shell-plate coverage are common (Pl. 2, fig. F), and as many as six openings have been seen in one individual. It would appear that the number of shell-plates in abnormal forms is roughly proportional to the number of apertures.

There is also a slight increase in the amount of organic cement binding the plates of the abnormal forms.

There appear to have been only two previous reports concerning the surface ultrastructure of *T. lineare*, both of which are based on carbon and metal-shadowed preparations. Thomas and Hovasse (1962) described two types of plates, large and small, with the aperture bordered by a collar of biconvex spines. Mercier *et al.* (1964) described the aperture as being surrounded by one or two circles of small spiny plates.

REPRODUCTION

An estimate of the doubling time was obtained by growing three replicate cultures, and recording the number of animals present at regular intervals. Growth curves produced from these results show that the doubling time is between 3·0 and 3·4 days. A similar calculation for *Euglypha rotunda* gave a doubling time of between 1·4 and 1·9 days (Hedley and Ogden, 1973).

Binary fission is completed in approximately 60 minutes. The initial stages are difficult to observe in *T. lineare* due to the small size of the animal and the oblique position of the aperture. Cytoplasmic division, once the daughter shell has been formed, proceeds in a manner similar to that previously described (Hedley and Ogden, 1973) for *E. rotunda*.

ULTRASTRUCTURE OF VEGETATIVE STAGE

The cytoplasm does not quite fill the shell cavity (Pl. 3, fig. A), but appears to be anchored to the apertural collar. Numerous fine processes can be seen extending between the cytoplasm and the inner shell wall (Pl. 3, fig. A). Unevenly spaced pellicular microtubules lie beneath the plasmalemma and run in an antero-posterior direction. The mitochondria are ovoid or spherical in shape, possess tubular cristae, and appear to be distributed at random throughout the cytoplasm.

Nucleus. The vegetative or interphase nucleus is usually spherical, between 4·4 and 5·7 μm in diameter, occupying a central position at the posterior end of the cytoplasm (Pl. 3, figs. A and D). It is bound by a nuclear envelope made up of two tripartite membranes, the outer membrane being continuous with the granular endoplasmic reticulum. The nuclear matrix is finely granular, with small concentrations of densely-staining chromatin scattered throughout and a dense nucleolus.

Although the nucleus during interphase was spherical in most animals examined, a number have been observed which are cone-shaped posteriorly (Pl. 4, fig. A), and it is assumed that this is correlated with the early stages of prophase. The cone-shape is caused by the convergence of numerous microtubules towards a specific region at the posterior end of the cytoplasm. The microtubules are first seen converging on the nucleus around its equatorial region (Text-fig. 1 ; Pl. 6, fig. A), whilst at a level in the region of the contractile vacuoles they lie in the endoplasmic reticulum at some distance from the nucleus. Although it is difficult to estimate the number of microtubules present because of the close relationship of the dense endoplasmic reticulum, it is apparent that the numbers increase to approximately 70 in the posterior region of the cytoplasm.

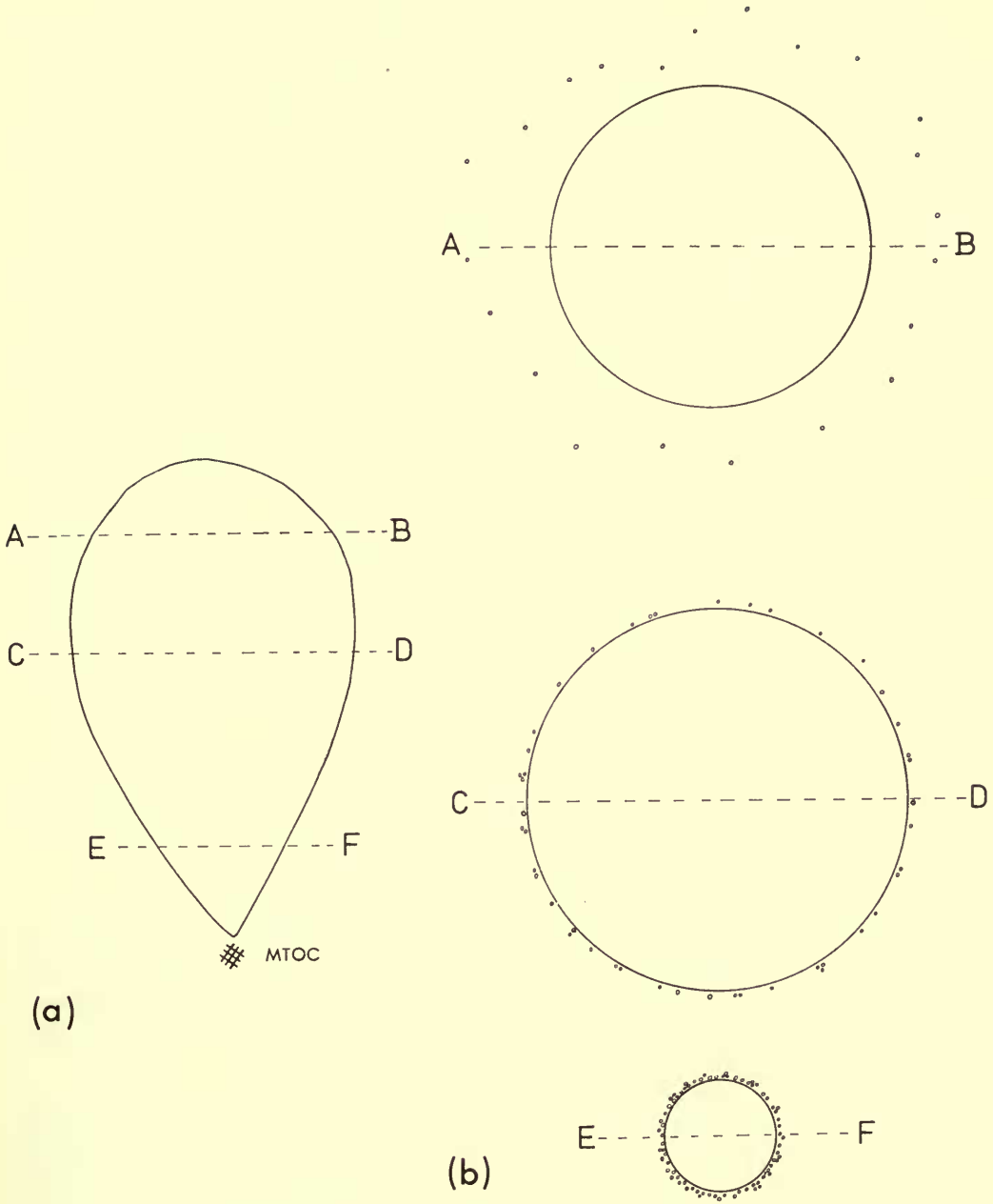

FIG. 1. (a) Diagram of a longitudinal section through the nucleus (see Pl. 4, fig. A). (b) Tranverse sections at those levels of the nucleus marked A-B, C-D and E-F in (a), to illustrate the microtubules converging towards the microtubule-organizing-centre (MTOC), as discussed in the text.

The microtubules lie close to the nuclear membrane (Pl. 4, fig. C), but do not lie in invaginations similar to those reported by Leadbeater and Dodge (1967) for the dinoflagellate *Woloszynskia micra*. They appear to attach to an electron-dense area close to the distal end of the nucleus (Pl. 4, figs. C and D), which is comparable with previously described and similar regions in the alga, *Chara* (Pickett-Heaps, 1968), fungal zoospores (Fuller and Calhoun, 1968), the soil amoeba, *Acanthamoeba castellanii* (Bowers and Korn, 1968), the marine amoebae, *Stereomyxa ramosa* and *S. angulosa* (Benwitz and Grell, 1971a, b), and the marine protist, *Labyrinthula* (Perkins, 1970). Such regions of attachment for microtubules are referred to in other cells under a variety of terms. For example, in dividing plant cells, Pickett-Heaps (1969) referred to it as a 'microtubule-organizing-centre' – MTOC, and suggested that such centres initiate and control the arrangement of microtubules. An MTOC was also described by McCully and Robinow (1972) in association with the nucleus during mitosis in yeasts. In *Labyrinthula*, Perkins (1970) described the granular aggregate as a 'protocentriole', while the term 'paracentrosome' was suggested by Manton *et al.* (1970) for the precursor material seen to accumulate near each pole at meiotic division in the marine centric diatom, *Lithodesium undulatum*. Fuller and Calhoun (1968) stated that the kinetosome of fungal zoospores is an unlikely 'organizing centre' but suggested that the electron-opaque material surrounding the proximal third of the kinetosome could be such a 'centre'. In mouse oocytes electron-dense fibrillar areas, close to the nucleus, from which microtubules radiate are referred to by Szollosi *et al.* (1972) as 'microtubule foci'. Evidence that such regions indicate the site of microtubule formation in many systems has been provided by Tilney and Goddard (1970) and Tilney (1971), who conducted experiments on the breakdown and reformation of microtubules in certain species of Heliozoa.

Contractile vacuoles. Two or three contractile vacuoles occur at the edge of the granular endoplasmic reticulum (Pl. 3, fig. D) in the region of the nucleus. These vacuoles are often surrounded by numerous vesicles which are associated with the lumen of the vacuole, and they discharge directly into the shell cavity.

Microbodies. Microbodies appear in all the specimens examined (Pl. 3, fig. B). They are ovoid or spherical in shape, varying in size between 0·30 and 0·55 μm, with a dense granular matrix surrounded by a single unit membrane. Tubular elements, between 18 and 26 nm in diameter, appear within the matrix (Pl. 4, fig. E), and as many as four tubules are apparent as cross-sections or loops in some microbodies.

The microbodies in *T. lineare* are similar to those reported in various Foraminifera by Hedley *et al.* (1967), Hedley and Wakefield (1969) and Febvre-Chevalier (1971), in possessing tubular-like elements within the matrix. Hedley and Wakefield (1969) imply that such organelles are possibly a normal component of the cytoplasm of the Foraminifera. The structure and function of microbodies has been reviewed recently by Hruban and Rechcigl (1969).

Endoplasmic reticulum. A concentrated mass of granular endoplasmic reticulum usually surrounds the nucleus, and appears more electron-dense than the surrounding cytoplasm due to the concentration of ribosomes (Pl. 3, figs. A and D). In early

prophase, however, the nucleus is drawn out distally from the endoplasmic reticulum region (Pl. 4, fig. A).

Pigment zone. A zone of large vacuoles lies immediately anterior to the endoplasmic reticulum region (Pl. 3, fig. A). They contain electron-dense material and have previously been equated by Hedley and Ogden (1973) to the 'pigment zone' of earlier light microscopy workers. A probable developmental sequence in the formation of the electron-dense material in the vacuoles is illustrated in Pl. 3, figs. A and B – the various stages are labelled 1 to 4. The initial stage (1) shows the matrix to be granular with a small electron-dense area in the centre. As this deeply stained area increases in size (2 and 3) the matrix becomes coarser and uneven rents appear in the electron-dense material. In the final stage (4) the vacuole is composed mainly of electron-dense material. The rents in the electron-dense material are possibly caused by its impermeability to the embedding resin. No explanation can be made at present regarding the occurrence of this constant structure, or of the occasional empty vacuoles seen here and reported previously by Hedley and Ogden (1973) for *Euglypha rotunda*.

Golgi apparatus. A single Golgi apparatus lies immediately posterior to the nucleus at the edge of the endoplasmic reticulum region (Pl. 4, fig. A). Both smooth and coated vesicles are associated with the Golgi saccules (Pl. 4, fig. B). In addition, the saccules of the Golgi are often distended by small concentrations of densely staining fibrillar material (Pl. 5, fig. C). These concentrations appearing in the outer saccules of the dictyosome become progressively spherical as the concentration of material increases and finally are detached at the margins of the saccules as membrane-bound vesicles. At this stage the fibrillar material is concentrated mainly at the centre of the vesicle, and has small strands radiating from the centre (Pl. 3, fig. C). The vesicles then pass around the outside of the endoplasmic reticulum region and are distributed randomly throughout the cytoplasm.

It has already been established (Favard, 1969) that the Golgi apparatus appears to play a role in the packing of secretory products for export and storage. The chemical constituents of such secretory products in both plants and animals are usually polysaccharide or protein macromolecules. Hedley and Wakefield (1969) suggested that the polysaccharide produced by the Golgi apparatus of the marine protozoon, *Gromia oviformis*, appeared to be used in the formation of the proteinaceous shell-wall. Schwab (1969) also suggested that the fibrillar shell-wall material in the marine foraminifera, *Myxotheca arenilega*, is produced by the Golgi apparatus. More recently, Hedley and Ogden (1973) have suggested that the fine fibrillar, polysaccharide material contained in the spherical, membrane-bound vesicles of *E. rotunda* might be utilized to form the proteinaceous cement or glue, that lines the inside of the siliceous shell and also binds the shell-plates of this testate amoeba.

It seems reasonable to suggest that the fibrillar vesicles (Pl. 3, fig. C) produced by the Golgi apparatus in *T. lineare* might be the initial stages in the formation of the organic cement bodies (Pl. 5, fig. E).

Another type of vesicle, with a double unit membrane and usually electron-transparent contents, often occurs lying in the area of cytoplasm between the distal

end of the granular endoplasmic reticulum region and the Golgi apparatus (Pl. 5, fig. D). Occasionally they occur in the cytoplasm anterior to the granular endoplasmic reticulum region, surrounded by a ring of smooth endoplasmic reticulum (Pl. 5, fig. A). The function of these vesicles is at present unknown.

Food particles. Food particles, which are usually gram-negative bacteria, occur throughout the cytoplasm, whereas in *E. rotunda* they are confined to the anterior third of the cytoplasm (Hedley and Ogden, 1973). Nevertheless, digestion in *T. lineare* probably occurs in the enlarged food vacuoles which are found normally in the anterior cytoplasm.

Reserve shell-plates. Reserve shell-plates are formed in that region of granular endoplasmic reticulum that surrounds the nucleus and close to the Golgi apparatus. As the shell-plates are formed they become closely packed together in a region just anterior to the nucleus (Pl. 6, fig. D). Both large and small shell-plates appear to be formed at the same time. The apertural plates appear to be the last to be formed and are usually seen only in the posterior region of the cytoplasm, with their dorsal teeth pointing outwards.

Large inclusions, containing electron-dense material, are present in the granular endoplasmic reticulum region (Pl. 3, fig. A). These inclusions appear to be less dense than the 'pigment zone' vacuoles and are often seen to fuse with the unit membrane of reserve shell-plates (Pl. 6, fig. B). It seems probable that these inclusions are associated with the formation of the siliceous plates as they are frequently seen in specimens containing reserve shell-plates. It is noted, however, that whereas in sections of animals fixed and subsequently treated in the absence of heavy metals the siliceous plates are naturally electron-dense (Pl. 7, fig. A), the inclusions in the endoplasmic reticulum are electron-transparent.

The only additional reference to those previously reported by Hedley and Ogden (1973) regarding the formation of siliceous material is that of Cachon and Cachon (1971) on silica metabolism in Radiolaria. They suggest that the siliceous shells of digested micro-organisms, upon which radiolarians feed, are used to produce their siliceous skeletons, and the unused siliceous material is rejected in gel form.

Organic cement. The organic cement or glue that holds the plates together is a fine fibrillar material probably produced by the Golgi apparatus. It is circulated within the cytoplasm in membrane-bound vesicles (Pl. 5, fig. E), and is discharged mainly in the anterior region where the vesicles fuse with the plasmalemma. In abnormal forms there is usually more cement at shell-plate junctions than in normal specimens.

Pseudopodia. The fine structure of the pseudopodia consists of ground plasm limited by a membrane, and occasionally containing microfilaments. Our observations are, however, limited to cytoplasmically joined individuals, in which microtubules are often seen in the cytoplasm internal to the aperture, but not externally (Pl. 6, fig. C). Similar observations on microtubules were reported for *Difflugiella* sp. by Griffin (1972) and for *E. rotunda* by Hedley and Ogden (1973).

Rosette groups. Rosette-like groups are frequently seen in clonal cultures which are four or five weeks old (Pl. 2, fig. G and Pl. 7, figs. C–E). The groups are usually

composed of five individuals or less and are joined by cytoplasmic connections. These connections contain numerous cement bodies and less frequently mitochondria (Pl. 7, fig. C). In contrast to the situation reported by Hedley and Ogden (1973), for similar formations in *E. rotunda*, microfilaments are seldom present. Individuals with reserve shell-plates are seldom seen in rosette groups, suggesting that such formations may be the result of starvation.

Abnormal forms

The cytoplasm of animals with abnormal shapes (Pl. 2) is similar to that of normal animals with the exception that the number of nuclei appears to be related to the number of apertures (Pl. 7, fig. F). From our observations all attempts by these forms to divide are abortive.

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PLATE 1

A. Aperture of *T. lineare* ; note the invagination produced by the curved shell-plates. × 7100
B. Apertural view of *T. lineare* showing the arrangement of large, circular shell-plates. × 2900
C. Two apertural plates ; the lower one shows a characteristic median fold. × 23 000
D. A preparation from which the organic cement has been removed illustrating the arrangement of apertural and small shell-plates. × 8650
E. Lateral view of *T. lineare* illustrating the oblique position of the aperture. × 2900
F. Individual shell-plates ; note the fold in small shell-plate (arrowed). × 11 500

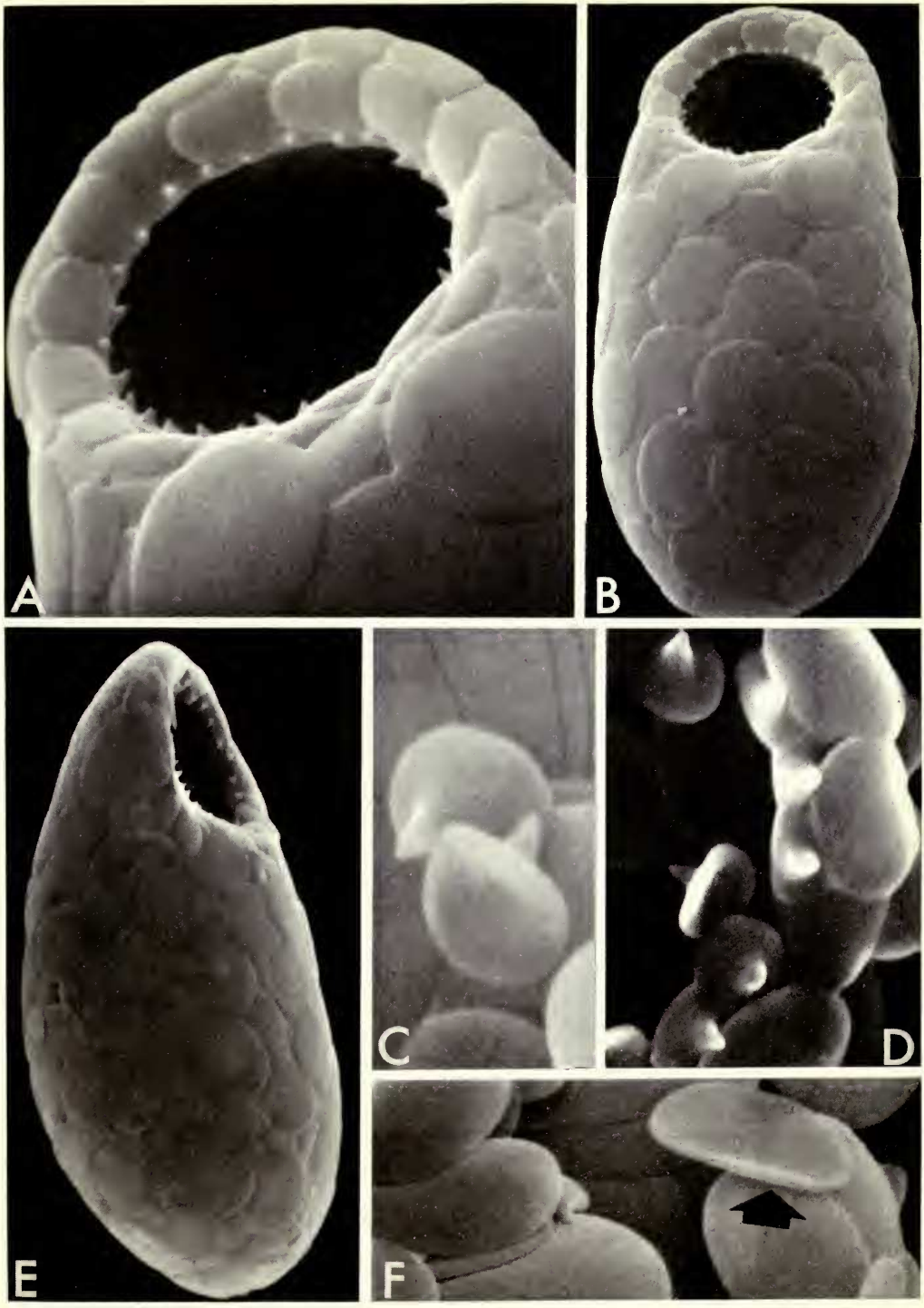
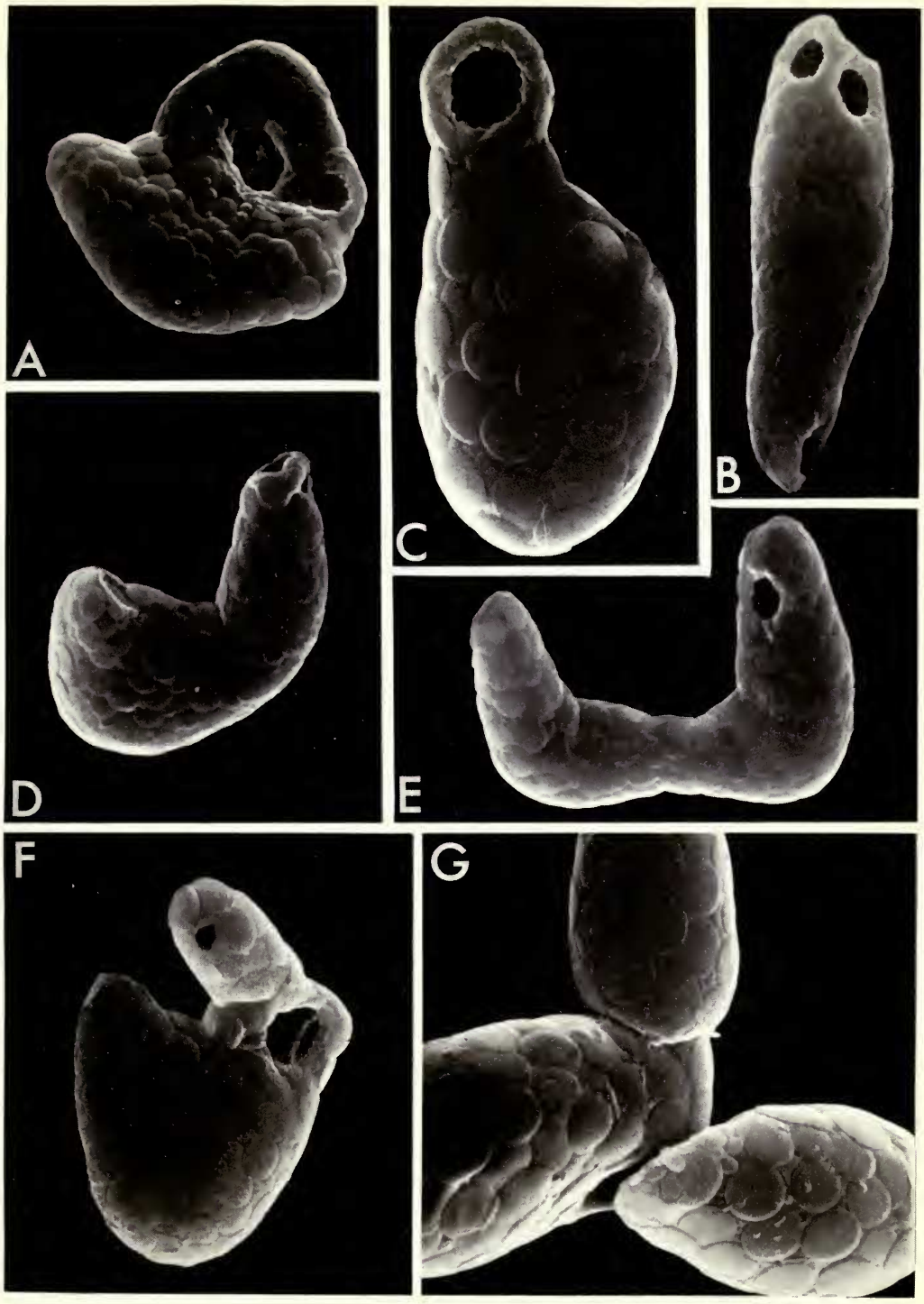

A
B
C
D
E
F

PLATE 2

A–F. Abnormal variants of *T. lineare* ; note that the apparent imbrications are due possibly to electrons penetrating the borders of the shell-plates, these are not seen when the accelerating voltage on the scanning electron microscope is reduced.
A, B, D, E and F × 1000 C × 2000

G. Groups of three animals in 'rosette' formation. × 1400


A
B
C
D
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G

PLATE 3

A. Longitudinal section showing the pigment zone (pz), electron-dense inclusion (i), granular endoplasmic reticulum (ger), nucleus (n) and Golgi apparatus (G). × 4300

B. Section showing various vacuoles containing bacteria (b), microbodies (m), and the probable stages in the development of electron-dense particles (stages 1–3) – stage 4 is shown in fig. A. × 15 950

C. Section showing vesicles containing fibrillar material (fm) concentrated in the centre with radiating strands. × 23 900

D. Transverse section showing three contractile vacuoles (cv) which are at systole, nucleus (n) and the concentrated mass of perinuclear endoplasmic reticulum (ger). × 7800

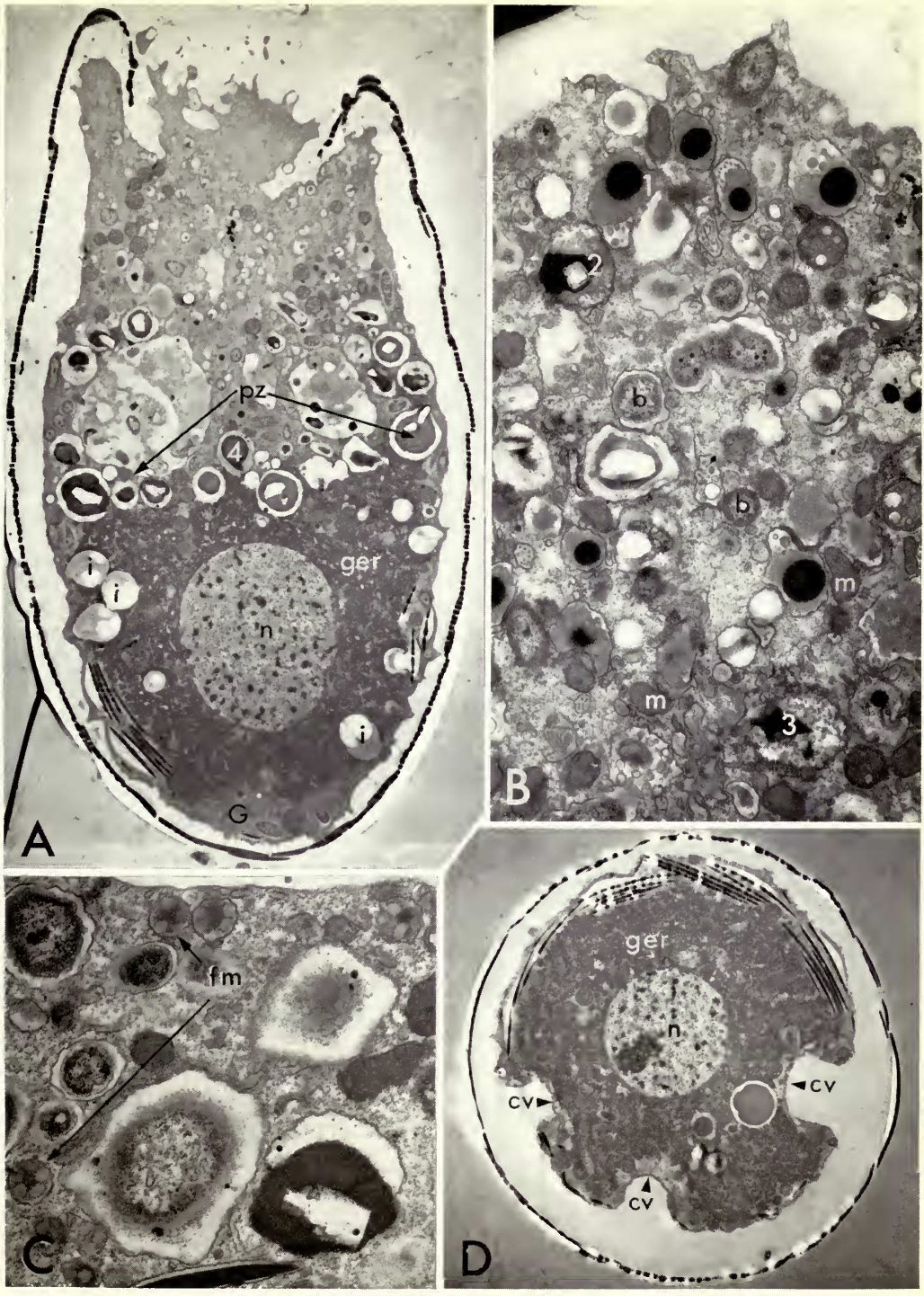
A
pz
4
ger
i
i
n
i
G
B
1
2
b
b
m
m
3
C
fm
D
ger
n
cv
cv
cv

PLATE 4

A. Longitudinal section showing the position and ovoid shape of the nucleus (n) at prophase, Golgi apparatus (G), the cytoplasmic attachments to the shell and the concentrated mass of perinuclear endoplasmic reticulum (ger.). × 7800

B. Cross-section of the posterior region of nucleus (n) showing proximity of numerous microtubules (arrowed), Golgi apparatus (G) and coated vesicles (cves). × 20 750

C. Section showing microtubule (mt) close to nuclear membrane (nm) and the terminal position of the microtubule-organizing-centre, MTOC (arrowed). × 30 100

D. Section immediately posterior to the nucleus showing nuclear envelope (ne) and numerous microtubules (mt) converging onto microtubule-organizing-centre, MTOC (arrowed). × 56 700

E. Section through two microbodies showing tubular elements. × 44 600

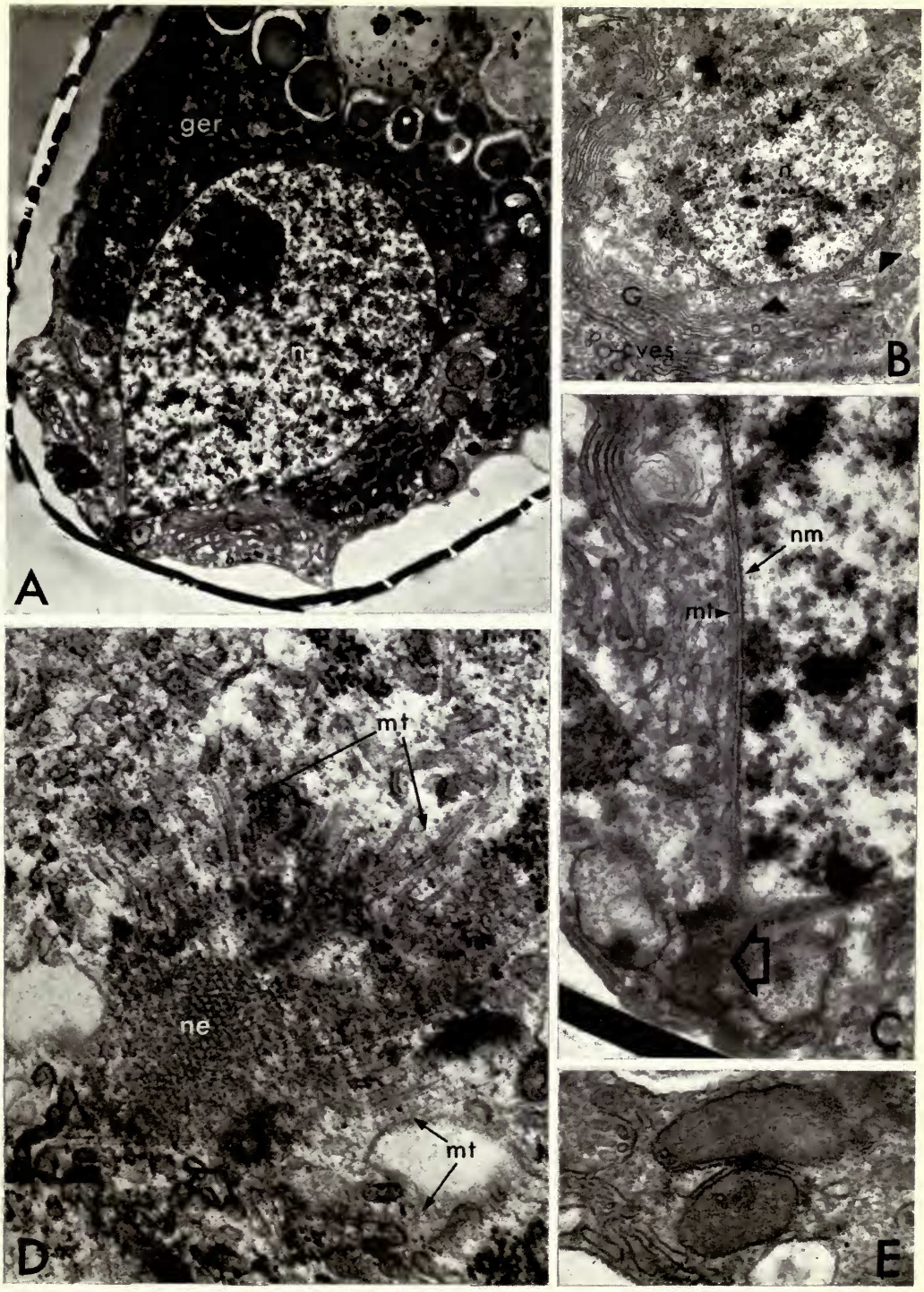
ger
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PLATE 5

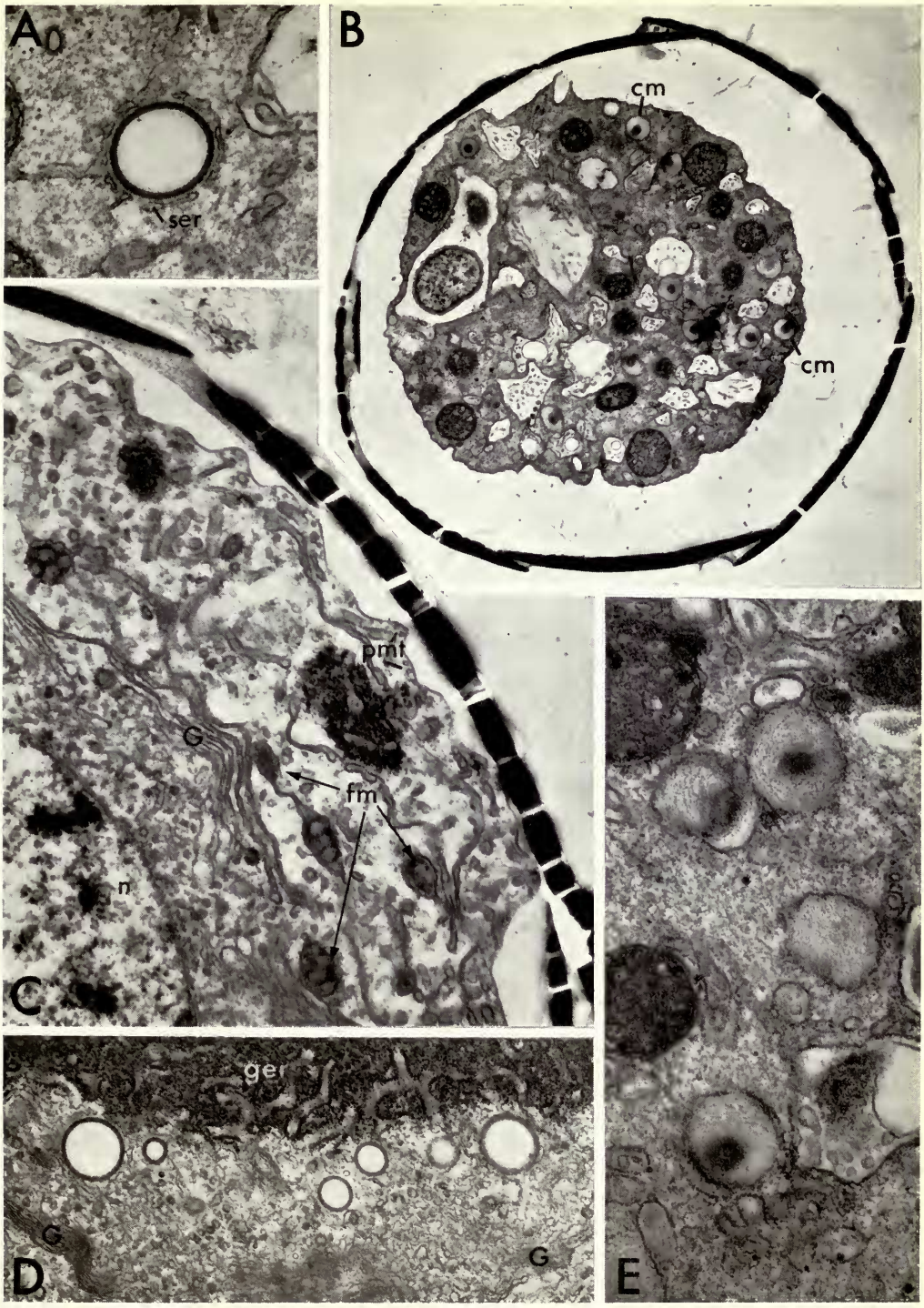
A. Vesicle with double-unit membrane and enclosed by smooth endoplasmic reticulum (ser) (see also fig. D). × 30 100

B. Transverse section through anterior region of cytoplasm showing numerous cement bodies (cm) and thin cytoplasmic strands in the space between cytoplasm and shell. × 10 400

C. Section showing Golgi apparatus (G) with concentrations of fibrillar material (fm) in the saccules, nucleus (n) and pellicular microtubules (pmt). × 22 300

D. Probable origin of electron-transparent vesicles in the region between granular endoplasmic reticulum (ger) and Golgi apparatus (G) (see also fig. A). × 20 050

E. Cement bodies with fibrillar matrix and electron-dense centres. × 40 100


A
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pmt
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ger
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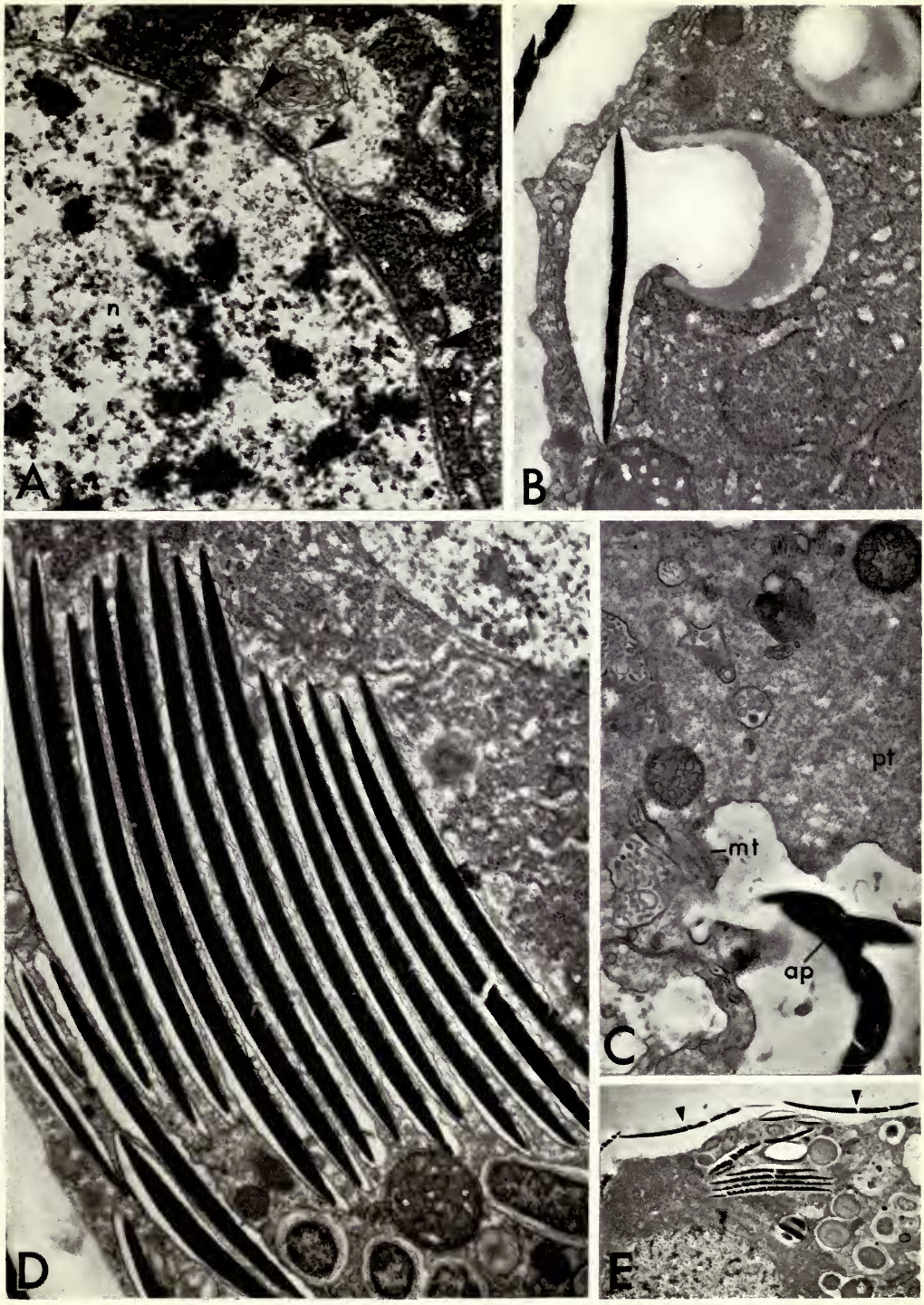
G
D
E

PLATE 6

A. Section through equatorial region of nucleus (n) showing surrounding microtubules (arrowed). × 30 100
B. Fusion of electron-dense inclusion with reserve shell-plate vesicle. × 22 300
C. Section through apertural region showing internal microtubules (mt), apertural plate (ap) and pseudopodial trunk (pt) which is relatively structureless. × 15 000
D. Stack of reserve shell-plates each in separate membrane-bound vesicle. × 22 300
E. Portion of shell showing shell-plates (arrowed) with concave surface outwards. × 4300


n
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B
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mt
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C
D
E

PLATE 7

A. Glutaraldehyde-fixed and unstained section showing the arrangement of reserve shell-plates. × 4300

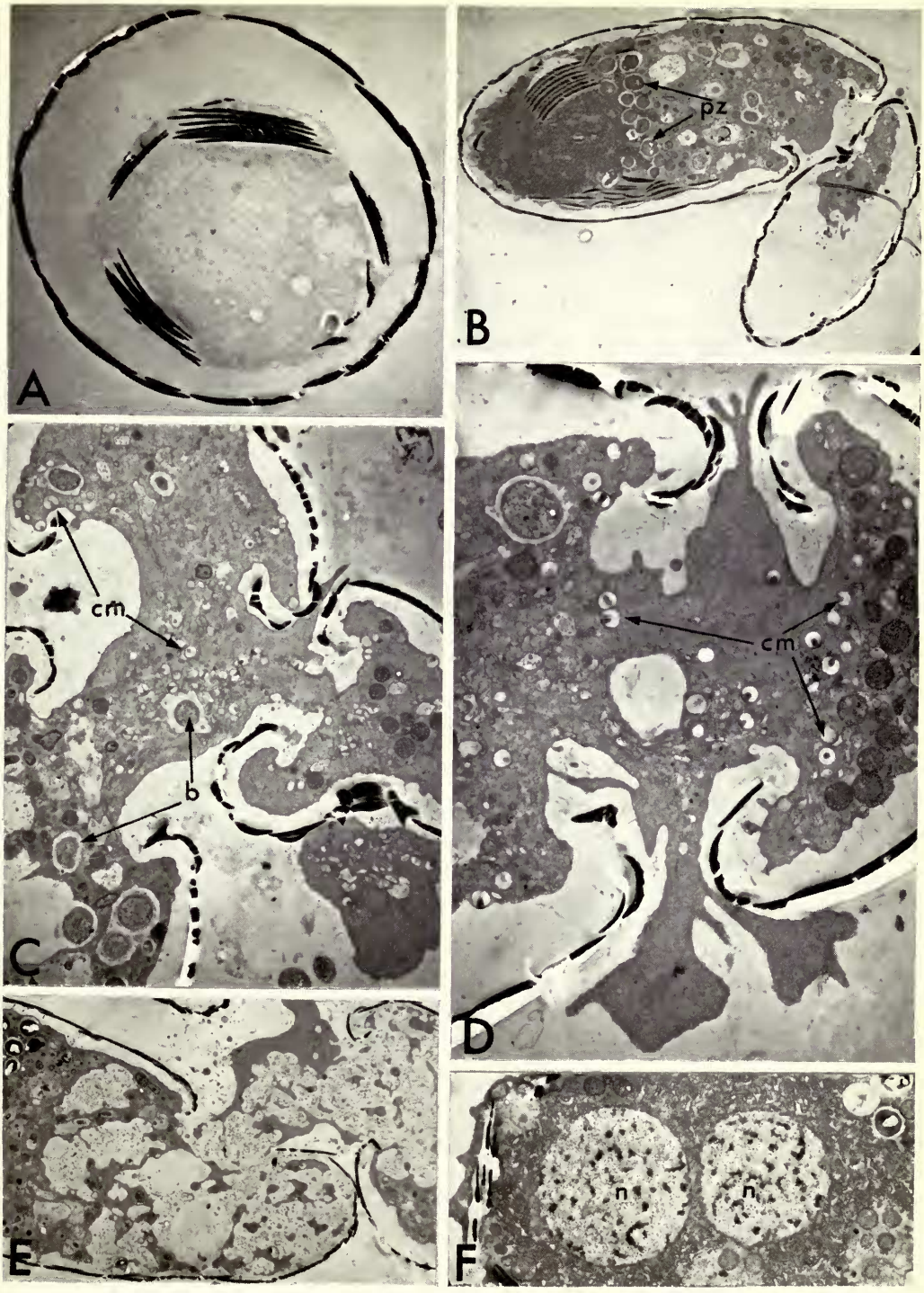
B. Section of two animals directly apposed ; note the larger individual contains numerous reserve plates and a well-defined pigment zone (pz). × 2100

C. Apertural region of a 'rosette' group of four individuals ; note the presence of vacuoles containing bacteria (b) and the numerous cement bodies (cm). × 4300

D. Section through two united adult specimens showing cement bodies (cm) and pseudopodial extensions ; note the structureless nature of the pseudopodia. × 7800

E. A 'rosette' group of three animals showing the diffuse nature of the cytoplasm. × 2800

F. Abnormal individual with two nuclei. × 5750


A
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